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THE MATING METHOD OF IDENTIFICATION OF A COPRINUS GROWING ON GERMINATING SEEDS OF MANGEL AND SUGAR-BEET.

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The Mating Method of Identification of a Coprinus growing on Germinating Seeds of Mangel and Sugar-beet.

BY

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With Plate XXVIII and six Tables in the Text.

I. Introduction.

IN 1914 Mr. Raymond Finlayson, of the Seed Testing Laboratory, Wood Green, London, observed a *Coprinus* coming up on germinating seeds [1] of Mangel, Beet, and Sainfoin, and he sent some of the material to Mr. Carleton Rea. Mr. Rea regarded the fungus as *Coprinus pilosus*, Beck,[2] but sent some of the seeds to the senior author (A. H. R. B.) with a request for his opinion. The *Coprinus* duly came up on some of the moistened seeds and from its morphological characters it was identified by Buller as *Coprinus lagopus*, Fr., and it was considered by him as identical with the form of *C. lagopus* which occurs very commonly on horse-dung both in England and Canada.[3]

In 1919 Pape [4] published a note on the occurrence of a *Coprinus* on germinating Mangel and Beet seeds. His attention had been called to the matter by Dr. Zade, of Jena. Pape observed that the fungus grew almost invariably on the seed-balls (pericarps) themselves, and he observed nothing to suggest that the fungus is a parasite. He says: 'The question of the significance of the fungus in respect to the Beet seed upon which it grows

[1] Mangel and Beet seeds, when sown, are enclosed by a rough pericarp. The *Coprinus* under discussion grows on the pericarp.

[2] Carleton Rea, *in litt.*

[3] A. H. R. Buller: Researches on Fungi, London, vol. iii, p. 308, 1924. The *Coprinus* obtained from the Mangel seeds is illustrated in Fig. 141, D, p. 320.

[4] Pape: *Coprinus* auf Rübensamen. Mitteilungen a. d. Biol. Reichsanst. f. Land- und Forstwirtschaft, Heft 17, pp. 13–16, 1919, with two illustrations.

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must be left open. A lowering of the germinating power of the seed due to the fungus or damage to the young seedling from it has not been observed. The fact that the fungus is most abundant upon seed which we found to have a low germinating power may be due to the fungus finding more nourishment in seeds which have failed to germinate than in those which produce seedlings.' Pape identified the fungus as *Coprinus nycthemerus*, Fr.

In 1926 the *Coprinus* was observed by Mr. W. A. Dillon Weston, of the School of Agriculture at Cambridge, England, coming up on Mangel seeds, and by Mr. M. W. Gardner, of the Agricultural Experiment Station at Purdue University, Lafayette, U.S.A., coming up on Sugar-beet seeds ; and each of these investigators sent a packet of his infected seeds to the senior author of this paper (A. H. R. B.) with a request for an identification of the fungus. Thereupon the work about to be described was undertaken.

New *Coprinus* fruit-bodies were obtained by the writers from (1) the Mangel seeds, and (2) the Sugar-beet seeds, by sowing the seeds on sheets of wet filter-paper in a crystallizing dish. Some of the seeds germinated normally, whilst others did not germinate at all. On the latter, in both cultures, after a lapse of about three weeks, several *Coprinus* fruit-bodies appeared (Pl. XXVIII). The *Coprinus* on the Mangel seeds was identical in appearance with the *Coprinus* on the Sugar-beet seeds, and both Coprini had all the morphological and growth characteristics of *Coprinus lagopus*, Fr., as described and illustrated in vol. iii of Buller's 'Researches on Fungi'.[1]

Coprinus lagopus is of very common occurrence on horse-dung balls both in England and in Canada, and it can almost always be procured by putting fresh horse-dung balls in a covered crystallizing dish and leaving them there for two or three weeks at room temperatures.

The recent advance in our knowledge of the sexual process in the Coprini permits of an experimental verification of such an identification as has just been made. The experimental method of identification may be called the *mating method* ; and, in what follows, it will be treated of both in theory and in practice.

II. The Mating Method.

When monosporous mycelia derived from spores of a *single* fruit-body of *Coprinus lagopus* are paired in all possible ways on dung-agar plates and the subsequent development or non-development of clamp-connexions is taken as a criterion of a positive or of a negative sexual reaction respectively, it is found that the mycelia fall into *four* sexual groups, which may be expressed in Mendelian symbols as follows: (AB), (ab), (Ab), and (aB).

[1] A. H. R. Buller: loc. cit., pp. 299–327, Figs. 130–47.

Thus Hanna[1] with ten monosporous mycelia from as many spores of a single wild fruit-body, which he paired in all possible ways, obtained the results embodied in Table I. In this table a (+) sign indicates that clamp-connexions appeared in the compound mycelium and a (−) sign that they did not. The numbers 50–59 were arbitrary numbers given to particular mycelia. After the results of pairing had been obtained, the table was rearranged so as to bring like mycelia together, and the Mendelian symbols required to explain the reactions were then added.

		AB			ab				Ab		aB
		51	52	54	55	57	58	59	50	56	53
AB	51	−	−	−	+	+	+	+	−	−	−
	52	−	−	−	+	+	+	+	−	−	−
	54	−	−	−	+	+	+	+	−	−	−
ab	55	+	+	+	−	−	−	−	−	−	−
	57	+	+	+	−	−	−	−	−	−	−
	58	+	+	+	−	−	−	−	−	−	−
	59	+	+	+	−	−	−	−	−	−	−
Ab	50	−	−	−	−	−	−	−	−	−	+
	56	−	−	−	−	−	−	−	−	−	+
aB	53	−	−	−	−	−	−	−	+	+	−

TABLE I. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia obtained from the spores of a single Vancouver fruit-body. A similar table is obtained when ten monosporous mycelia derived from ten spores of one fruit-body are paired in all possible ways with ten monosporous mycelia derived from ten spores of another fruit-body, provided the two fruit-bodies belong to one and the same geographical strain.

In Table I it will be observed that the ten monosporous mycelia fall into *four* groups.

When monosporous mycelia derived from spores of a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with monosporous mycelia derived from spores of another fruit-body of the *same* geographical strain, it is found that, as before, the mycelia fall into four sexual groups to which may be given the Mendelian symbols (AB), (ab), (Ab), and (aB). This was proved by Hanna,[2] whose table of results for matings of the kind under discussion resembles Table I.

When monosporous mycelia derived from a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with the monosporous mycelia of *another* geographical strain, perfect fertility

[1] W. F. Hanna: The Problem of Sex in *Coprinus lagopus*. Ann. Bot., vol. xxxix, p. 436, 1925.
[2] Ibid., p. 443.

results, i. e. clamp-connexions appear in every plate. Thus Hanna [1] mated eleven monosporous mycelia, derived from as many spores taken from an Edmonton (Province of Alberta) fruit-body, in all possible ways with eleven monosporous mycelia derived from as many spores taken from a Winnipeg (Province of Manitoba) fruit-body, with the results shown in Table II.[2]

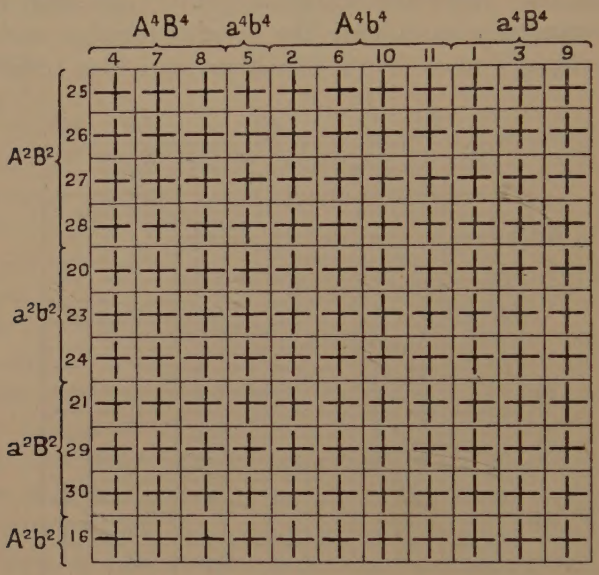

TABLE II. *Coprinus lagopus*. The pairing of eleven monosporous mycelia of an Edmonton fruit-body (No. 2) with eleven monosporous mycelia from a Winnipeg fruit-body (No. 4).

Table II contains nothing but (+) signs, which indicates that clamp-connexions appeared in every pairing.

When monosporous mycelia derived from the spores of a fruit-body of *one species* of *Coprinus* are paired with monosporous mycelia derived from the spores of *another* morphologically distinct species of *Coprinus*, there is complete infertility. Thus the authors made pairings between *Coprinus lagopus* and *C. macrorhizus*, between *C. lagopus* and *C. Rostrupianus*, and between *C. Rostrupianus* and *C. macrorhizus*, with the results shown in Tables III, IV, and V respectively.

Tables III, IV, and V contain no (+) signs, thus showing that no clamp-connexions were developed in any of the pairings. The absence of

[1] Loc. cit., p. 441.

[2] Similar results have been recorded for *Schizophyllum commune* (Kniep), *Coprinus Rostrupianus* (Newton), *C. comatus* (Brunswik), *C. radians* (Vandendries), and *Panæolus campanulatus* (Vandendries); but, recently, Vandendries has found an exception to this rule in some strains of *Coprinus micaceus* which were sterile with one another (R. Vandendries, L'hétérohomothallisme et la stérilité entre races géographiques de *Coprinus micaceus*, in Mém. de l'Acad. roy. de Belgique, tom. ix, pp. 42–50, 1927).

clamp-connexions clearly indicates a failure in the attempt to produce *Coprinus* hybrids.

The difference in the results obtained by mating (1) monosporous mycelia of *Coprinus lagopus* derived from a single fruit-body or from two fruit-bodies of one and the same geographical strain, (2) monosporous mycelia of *Coprinus lagopus* derived from two geographical strains, and

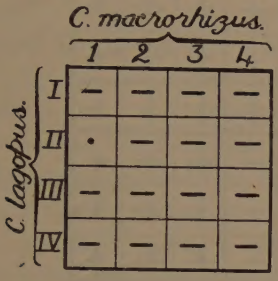

TABLE III. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

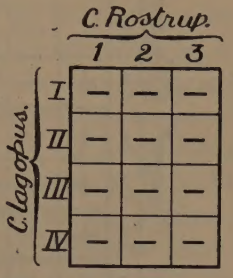

TABLE IV. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with three monosporous mycelia of *C. Rostrupianus*, Hansen.

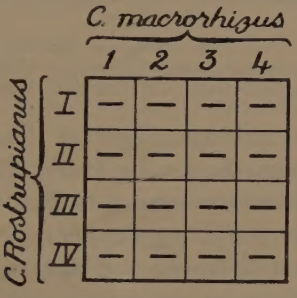

TABLE V. The pairing of four monosporous mycelia of *Coprinus Rostrupianus*, Hansen, with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

(3) monosporous mycelia derived from two morphologically distinct species of *Coprinus* has now been set forth. Using this difference as a guide, an attempt was made to determine whether or not the *Coprinus* which occurs on Mangel seeds at Cambridge, England, and on Sugar-beet seeds at Lafayette, U.S.A., are geographical strains of one and the same species, and, in particular, of *Coprinus lagopus* as it occurs on horse-dung at Winnipeg, Canada.

III. EXPERIMENTAL RESULTS.

Three monosporous mycelia were obtained from three spores derived from each of the following: (1) a *Coprinus* fruit-body which grew on Cambridge Mangel seeds; (2) a *Coprinus* fruit-body which grew on Lafayette Sugar-beet seeds; and (3) a *Coprinus lagopus* fruit-body which

came up spontaneously on horse-dung gathered at Winnipeg. These nine monosporous mycelia were then paired on dung-agar plates in all possible ways, with the results embodied in Table VI.

		C. lagopus			*Mangel*			*Sugar-beet*		
		1	2	3	1	2	3	1	2	3
C. lagopus	1	−	−	+	+	+	+	+	+	+
	2	−	−	−	+	+	+	+	+	+
	3	+	−	−	+	+	+	+	+	+
Mangel	1	+	+	+	−	−	−	+	+	+
	2	+	+	+	−	−	−	+	+	+
	3	+	+	+	−	−	−	+	+	+
Sugar-beet	1	+	+	+	+	+	+	−	−	−
	2	+	+	+	+	+	+	−	−	+
	3	+	+	+	+	+	+	−	+	−

TABLE VI. The pairing of nine monosporous mycelia, three derived from a Winnipeg *Coprinus lagopus* fruit-body, three derived from a Cambridge Mangel-seed *Coprinus* fruit-body, and three derived from a Lafayette Sugar-beet-seed *Coprinus* fruit-body.

In Table VI, in the pairings *Coprinus lagopus* × *Coprinus lagopus*, Mangel × Mangel, and Sugar-beet × Sugar-beet, the results, as was to be expected, are essentially like those shown in Table I; but the pairings were too few to give all the four possible groups of mycelia (AB), (ab), (Ab), (aB). However, in the pairings of *C. lagopus* × *C. lagopus* and Sugar-beet × Sugar-beet, it is obvious that three of the four possible groups of mycelia are represented (cf. Table I). In the pairings Mangel × Mangel, since no clamp-connexions appeared in any pairing, the three mycelia may have all belonged to one of the four possible groups, or one of the mycelia may have belonged to one group, e. g. (AB), and the two other mycelia to another group, e. g. (Ab) or (aB).

In Table VI complete fertility is shown in all of the following pairings: *Coprinus lagopus* × Mangel, *Coprinus lagopus* × Sugar-beet, and Mangel × Sugar-beet. These results, which resemble those of Table II, indicate that the *Coprinus* occurring at Cambridge, England, on Mangel seeds, the *Coprinus* occurring at Lafayette, U.S.A., on Sugar-beet seeds, and *Coprinus lagopus* occurring on horse-dung at Winnipeg, Canada, are merely three so-called geographical strains of one and the same species, namely, *Coprinus lagopus*, as described in vol. iii of Buller's 'Researches on Fungi'.

IV. Discussion.

As we have seen, the *Coprinus* on Mangel seeds was regarded by Mr. Carleton Rea as *C. pilosus*, Beck, and by Pape as *C. nycthemerus*, Fr., whereas we identify it as *Coprinus lagopus*, Fr., as very fully described and illustrated with numerous photographs and drawings in Buller's 'Researches on Fungi'.[1]

It seems highly probable that *Coprinus pilosus* and *C. nycthemerus*, as understood by Rea and Pape respectively, are but synonyms for *C. lagopus*, as understood by us. Pape [2] gives not only some illustrations but also a description of his fungus which enables us to perceive that his fungus and ours are in all probability one and the same species, and he adds: 'The foregoing characters of the fungus point to *Coprinus nycthemerus*, Fr., as being nearest to it. At the same time, a comparison between the present fungus and the figures given by Brefeld and Cooke of *C. nycthemerus* leave it uncertain whether the two are actually identical.' The diversity of opinion as to what the Mangel-seed fungus should be called is doubtless due to the fact that the original descriptions of many of the smaller Coprini are so brief and incomplete as to be little more than useless for identification purposes. Exactly what species of *Coprinus* are to be identified with *C. pilosus* and *C. nycthemerus* is not clear to the writers. *Coprinus pilosus* may well be a synonym for *C. lagopus*. The illustrations of *C. nycthemerus* as given by Brefeld,[3] Cooke,[4] and Ricken [5] do not resemble one another and do not suggest the *Coprinus* shown in our photographic illustration. Fries [6] and Rea [7] state that *C. nycthemerus* is uncommon, and Lange [8] in his monograph of the Danish Coprini does not even mention it. On the other hand, *Coprinus lagopus*, as understood by us, is an exceedingly common fungus in both Europe and North America. One of us (A. H. R. B.) has observed it many times on the under side of horse-dung plats in fields in England, and the ease with which the fungus may be obtained in fresh horse-dung cultures in both England and Canada goes to show that its spores must be widely dispersed in the pastures of both these countries.

As we have seen, the mating method has enabled the writers to determine with certainty that three Coprini obtained from as many different countries belong to one and the same species. There seems no reason why

[1] A. H. R. Buller: loc. cit.
[2] Pape: loc. cit., pp. 14–15.
[3] O. Brefeld: Untersuchungen über Pilze, Leipzig, Heft viii, 1909, Pl. XII, Figs. 10, 11.
[4] M. C. Cooke: Illustrations of British Fungi, London, Pl. 682, B.
[5] A. Ricken: Die Blätterpilze, Leipzig, 1915, Taf. xxi, Fig. 5.
[6] E. Fries: Hymenomycetes Sueciae, Upsala, 1857, p. 466.
[7] C. Rea: British Basidiomycetae, Cambridge, 1922, p. 511.
[8] J. E. Lange: Studies in the Agarics of Denmark. Dansk Botanisk Forening, 1915, Part II, *Coprinus*, pp. 32–50.

the same method should not be used to establish the identity of other heterothallic Hymenomycetes, physiological experiment thus directly helping the systematist.

V. Summary.

1. As shown by comparative morphology and by mating experiments, the *Coprinus* which comes up on germinating Mangel seeds in England and on germinating Sugar-beet seeds in the United States of America is identical with *Coprinus lagopus*, Fr., as it occurs on horse-dung in Canada.

2. *Coprinus lagopus* is not confined to a substratum of seeds, for it is a fungus of wide occurrence on horse-dung in pastures both in Europe and North America.

3. The mating method may be used to assist the systematist in deciding specific identity in the heterothallic Hymenomycetes in general.

EXPLANATION OF PLATE XXVII.

Illustrating the paper of Professor A. H. R. Buller and Miss D. E. Newton on the Mating Method of Identification of a *Coprinus*.

Photograph of fruit-bodies of *Coprinus lagopus* growing on Sugar-beet seeds (fruits) obtained from Lafayette, Indiana, U.S.A., about three weeks after the seeds had been placed on wet filter-paper in a crystallizing dish. Some of the seedlings have developed normally. Natural size.

Huth coll.

BULLER & NEWTON—IDENTIFICATION OF COPRINUS.

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